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Oil-in-water Pickering Emulsions Stabilized with Starch Particles and Formulated with Olive Oil: Colloidal Properties and Stability as Affected by Olive Oil Phenolic content

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In the last years, research has focused on the technological properties of olive oil polyphenols which have been proven to exert surface activity and emulsifying capacity in both model and real emulsified food matrices; however, to date, the effect of olive oil polyphenols in Pickering emulsions has not been explored yet. Aim of the work was to study the colloidal properties and physical stability of oil-in-water (O/W) Pickering emulsions made with esterified corn starch particles as emulsifiers and olive oil characterized by different polyphenols content (low, medium and high). Emulsions (30 % v/v oil) were obtained by using high pressure (HPH) homogenization (55 bar, 5 min). Colloidal properties (droplet size and distribution), stability (e.g. creaming index), rheological properties (flow curves and frequency sweep test), and microstructure of the emulsions were evaluated.
Starch particles and HPH allowed the formation of finely dispersed emulsions with droplet sizes with a median $d_{3,2}$ of 13±2 µm. Olive oil polyphenols affected significantly the colloidal properties of the emulsions as olive oils with higher polyphenols content showed narrower droplets distribution. All emulsions showed a non-Newtonian shear thinning behavior; moreover, samples with higher polyphenols content showed lower apparent viscosity. The Creaming Index (CI) indicated a good physical stability for all the systems with no phase separation over 30 days of storage, while emulsions made with oil with higher polyphenols content showed a lower CI confirming a positive effect of the bioactive in the stabilization of o/w emulsions. The results of this study highlight the feasibility of the preparation of O/W Pickering emulsions made of olive oils and the positive role of polyphenols on their colloidal and physical properties.

1. Introduction

Thanks to their role in developing lipid-containing matrices and in providing desirable appearance and mouthfeel properties, oil-in-water (O/W) emulsions are amongst the structural entities in a wide range of food and non-food (e.g. pharmaceutical and cosmetic) products. Due to their physical and thermodynamic properties the development of emulsified systems requires the understanding of the mechanisms of stabilization of the dispersed state and those causing their degradation. In the last decade studies focused on the stabilization mechanism of oil/water interface by solid particles (Pickering emulsions) which adsorb onto the interfacial layer with high absorption energy and act as physical barrier, thereby a higher stability could be achieved as compared to the traditional O/W emulsions, stabilized by surfactant and biopolymers (Yang et al., 2017). Moreover, in traditional emulsions the use of low molecular weight surfactants poses the treat of toxicity, poor bioavailability, foaming and biological interactions with other compounds (Berton-Carabin & Schroën, 2015) while Pickering emulsions are obtained by surface-active natural biopolymers that make these systems more acceptable in the food and pharmaceutical industry (Dickinson, 2012).
Recent researchers focused on the use of starch as a potential natural emulsifier in Pickering emulsions thanks to its non-toxicity (Ge et al., 2017), the high availability in nature (Li et al., 2019), low cost and high bioavailability (McClements & Gumus, 2016). However, native starches are not good emulsifiers and, thus, they are modified

by different procedures such as dry heating or chemical modification by esterification, being the latter the most commonly used (Khan & Ahmad, 2013). In the esterification process an acid or its derivative is used in order to replace the hydroxyl groups of starch which ester groups hence making it more hydrophobic (Fang et al., 2002) and increasing its emulsifying capacity.
Olive oil is one of the highly recommended lipid and is of crucial importance in the Mediterranean diet (López-Miranda et al., 2010) for several health benefits related to the peculiar fatty acid composition, the presence of bioactive phytochemicals and phenolic compounds, such as squalene and hydroxytyrosol (Stark & Madar, 2002). Besides being powerful antioxidants that can deeply affect the oxidative stability of foods, olive oil polyphenols are also compounds that can exert important technological functionality in both emulsified and gelled systems, directly or by interacting with other compounds such as proteins (von Staszewski et al., 2014). Several studies have been carried out to unravel the effect of polyphenols on the colloidal properties and stability of O/W emulsions (Di Mattia et al., 2014; López-Miranda et al., 2010; von Staszewski et al., 2014) while, to the best of the authors' knowledge, to date no studies are present in literature on the role of olive oil polyphenols in Pickering emulsions stabilized by starch particles.
The aim of this study was to investigate the effect of olive oil polyphenols in Pickering emulsions prepared with corn starch esterified with citric acid as emulsifier. In a preliminary step the effect of citric acid esterification on native corn starch was studied; then, the esterified corn starch was used to prepare the O/W Pickering emulsions by high pressure homogenization (HPH) and olive oils with different polyphenols content as dispersed phase. The emulsions were characterized for their colloidal and rheological properties, as well as physical stability.

2. Material and Methods

2.1 Material

Commercial corn starch (average droplet size: 90 μm) was purchased from a local company in Abruzzo (Italy). Two extra-virgin olive oils were purchased from a local market (IT and CO) while a refined olive oil (SI) was obtained from Sigma-Aldrich (Steinheim am Albuch, Germany). Oil samples were kept under dark conditions and at 15° C to avoid oxidation. All experiments were carried out by using oils from the same batch. Anhydrous citric acid with 99.5 % purity was purchased from Sigma- Aldrich (Steinheim am Albuch, Germany). All other chemicals used were of analytical grade and were from Sigma-Aldrich (Steinheim am Albuch, Germany).

2.2 Methods

2.2.1 Esterification of corn starch with citric acid

The method of (Kim et al., 2017) with some modification was used for the esterification of the native corn starch. Briefly, 30 % citric acid was dissolved in 50 ml distilled water and pH adjusted to 3.5 using 10 M NaOH. An aliquot of 50 g of corn starch was added in the citric acid solution under continuous mixing and the mixture was then left at room temperature for 16 h and after that it was dried in a hot air oven at 60 °C for 3-4 h till the 5-10 % (w/w) moisture content was achieved. After that it was again dried at 140 °C for 4 h. The dried starch citric acid mixture was grounded, washed with distilled water three times to remove any leftover citric acid and dried at 45 °C for 24 h. The powder was then finely ground by ball miller and sieved through a 90-micron sieve.

2.2.2 Scanning Electron Microscopy

Scanning electron microscopy was performed to characterize the microstructure of the native and esterified starch at 5 kV. About 100 mg of sample with double sided adhesive tape was mounted on the microscope (Leica, Cambridge, UK) stub and coated with gold.

2.2.3 Determination of total polyphenolic content of oils

Polyphenols were preliminarily extracted from olive oils according to the method of (Pirisi et al., 2000). Briefly, an aliquot of 2 g of oil was inserted in a centrifuge tube and a mixture of 1 ml n-hexane and 2 ml of methanol/water (60/40 v/v) was added and mixed 2 min by vortex. The dispersion was then centrifuged at 3000 rpm for 2 min. After removal of the methanol layer the extraction was repeated twice. Finally, all the methanol extracts were combined and washed twice with n-hexane. The n-hexane was then discarded and the methanol extract dried under reduced pressure at 30 °C.
The total phenolic content was determined by the Folin–Ciocalteu method (Singleton & Rossi, 1965). Standard curve was prepared using gallic acid and the phenolic content in the samples is expressed as mg of gallic acid/kg sample. The refined olive oil (SI) contained 1.51±0.00 mg/kg, CO and IT samples contained 285±1 mg/kg and 580±1 mg/kg of total polyphenols, respectively.

2.2.4 Preparation of O/W Pickering emulsions

A two-steps emulsification process was used. Initially an aqueous starch suspension (9.5 %w/v) was firstly mixed with a rotor-stator device (DI 25 basic, IKA, Stufen, Germany) at 20,000 rpm for 2 min with olive oil in a 70:30 (v/w) water/oil ratio. Then the coarse emulsion was subjected to high pressure homogenization (HPH) (Panda Plus 2000, GEA, Parma, Italy) at 55 bar with a circulation time of 5 min.

2.2.5 Droplet size distribution of O/W emulsions

The droplet size and its distribution of emulsions was measured by laser diffraction (Mastersizer 3000, Malvern Instruments Ltd. Malvern, UK) with a refractive index 1.33 for water and 1.59 for olive oil. The volume weighted (D4,3) parameters were used for analyzing the particles size distribution.

2.2.6 Rheological properties of O/W emulsions

Flow curves of Pickering emulsions were obtained by using a rheometer (MCR 302, Anthon Paar, Graz Austria). The steady shear properties of Pickering emulsions were carried out at 20°C using concentric cylinders (cup diameter = 28.92 mm and rotor diameter 26.66mm) at a continues shear rate of 1-300 1/s.

2.2.7 Optical Microscopy

Microscopic observation of Pickering emulsion was made by using Olympus-BX53 light microscope and images taken at different magnification (100 X and 400 X) by a 12-bit digital camera (QIQAM Fast 1394, Surrey, Canada). Before analysis starch was stained with Lugol's solution.

2.2.8 Physical Stability

The physical stability was carried by analyzing the variation of the particle size and its distribution of the samples kept at room temperature for 30 days. The emulsions were also photographed at regular intervals and Creaming Index (CI) was observed during the storage time.

2.2.9 Statistical analysis

All experiments were performed at least in triplicate. The analysis of variance (ANOVA) of the data was performed using SPSS version 24 (SPSS, Chicago, USA). Tukey's test was used for reporting the significance difference results at 95 % confidence level (P>0.05).

3. Results and Discussion

In a preliminary step, native corn starch was submitted to esterification in order to increase its emulsifying capacity; then, the colloidal properties and physical stability of Pickering emulsion stabilized with esterified starch and formulated with olive oils with different phenolic content were studied.

3.1 Effect of esterification on corn starch

Scanning Electron Microscope (SEM) were used to investigate the morphological changes of native corn starch due to esterification (Figure 1). It could be noticed that the outer surface of the native starch (NS) (Figure 1A) is dense, uniform, and smooth while that of the esterified starch (ES) ones (Figure 1B) is rough and grooves are clearly visible. These modifications could be attributed to the break down effect of the acid hydrolysis, as previously reported by other authors (Alimi & Workneh, 2018).

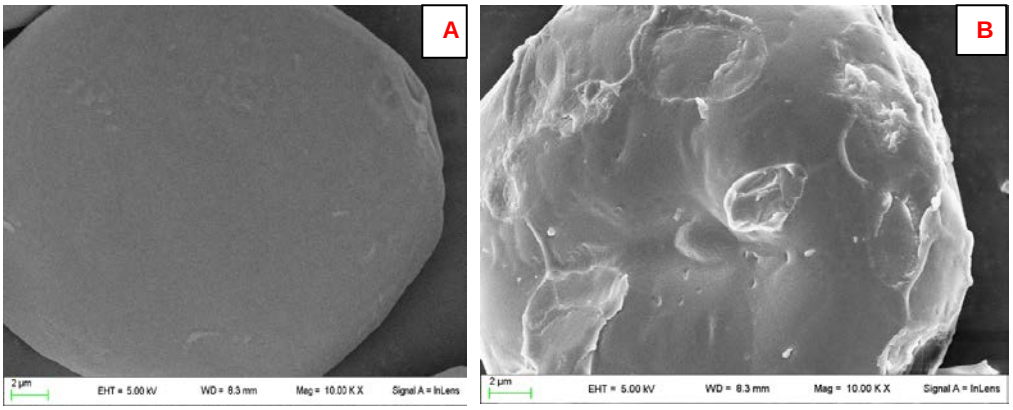

Figure 1: SEM images of corn starch granules (A- Native starch, B-Esterified starch)

3.2 Droplet size distribution of O/W Pickering emulsions

The 30% o/w Pickering emulsions were prepared using, as continuous phase, an aqueous 9.5% suspension of ES and two extra virgin olive oils, CO and IT, characterized by a significant different total phenolic content and equal to 285±1 and 580±1 mg/kg respectively; control emulsions were made with refined olive oil (SI) with negligible phenolic content. The Pickering emulsions prepared with native corn starch readily separated after preparation and hence were not taken into consideration for further experimentation.
In Figure 2A the droplet size distribution of Pickering emulsions prepared with the two extra virgin olive oils and the refined olive oil (SI) are shown. All the systems were characterized by a bimodal distribution, with a main population centered at around 40 μm and another population centered on smaller droplet sizes (4 μm); in the control systems, a broader distribution can be observed and the occurrence of a third population with a higher droplet sizes, was observed. These trends found correspondence with the volume mean diameter ($D_{4,3}$) whose data are shown in Figure 2B. In particular presence of phenolic compounds, the emulsions showed a droplet size significantly ($P<0.05$) lower as compared to the refined SI sample where no phenolic compounds are present. These results are in line with other literature studies on emulsified systems in which the presence of phenolic compounds allowed the formation of o/w emulsions with lower droplet size and enhanced physical stability (Di Mattia et al., 2014; von Staszewski et al., 2014)

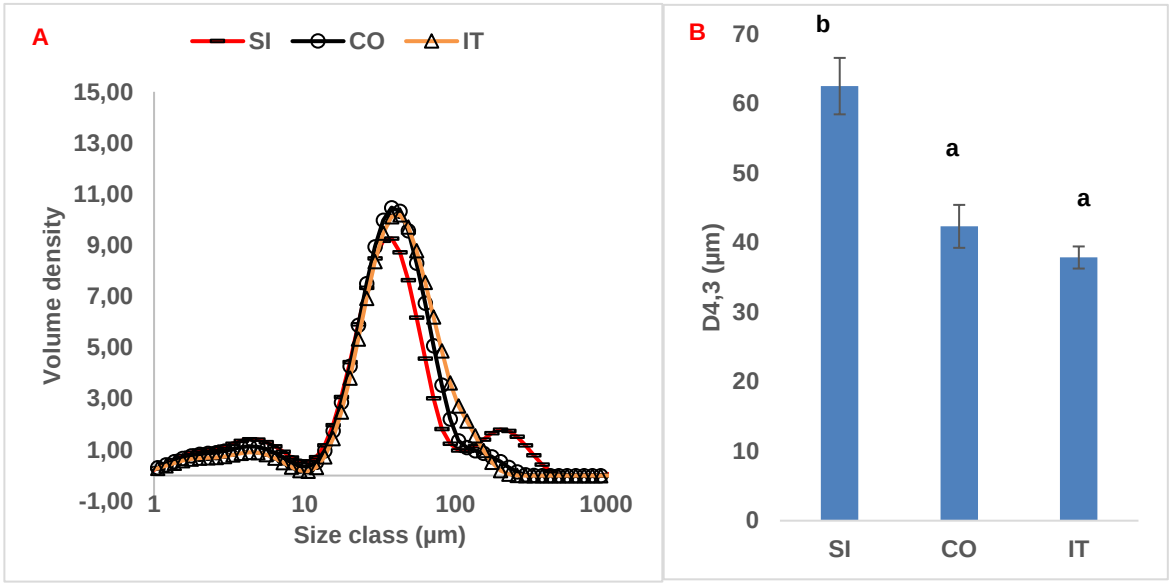

Figure 2 Size class distribution (A) and droplet size (B) of Pickering emulsions prepared with refined olive oil (SI) and two olive oils (CO and IT)

3.3 Effect of olive oil polyphenols on the flow behavior of O/W Pickering emulsions

The viscosity graphs of the o/w Pickering emulsions prepared with the different olive oils are shown in Figure 3. In general, at higher shear rate, the shear stress is increased, reflecting a non-Newtonian shear thinning behavior of the emulsions (data not shown) that could be attributed to the weak droplet association and the disruption of the tangled polymers whose breaking rate was higher than the rate of reformation which resulted in lower intermolecular resistance and reduction of samples viscosity (Ye et al., 2017). The presence of phenolic compounds lowered the apparent viscosity of CO and IT (Figure 3) in comparison to the refined SI sample in which no phenolic compounds are present.
As far as the rheological properties are concerned, the frequency sweep test evidenced a viscoelastic behavior which was affected by the total polyphenolic content as CO and IT samples showed higher G' and G'' values as compared to the refined SI olive oil sample (data not shown).

3.4 Optical microscope images and stability of Pickering emulsions

In Figure 4 the optical microscope images taken at two magnifications (4A: 100x, 4B: 400x) of the O/W Pickering emulsions are shown. The starch granules, stained with Lugol's solution, appeared in black color under the microscope. It could be noticed that oil droplets are surrounded and immobilized by the starch particles confirming the Pickering stabilization behavior.

The stability study for 30 days indicated that Pickering emulsions with higher phenolic content were more stable to creaming (Figure 5 A and B) and this trend is confirmed also by the Creaming Index (CI) (data not shown) data of IT and CO samples whose value was significantly lower ($P<0.05$) than that of the SI sample. The stabilizing effect of olive polyphenols was reported in literature by other authors that observed that at higher concentration of polyphenols a higher and longer stability of emulsions occurred(Caporaso et al., 2016).

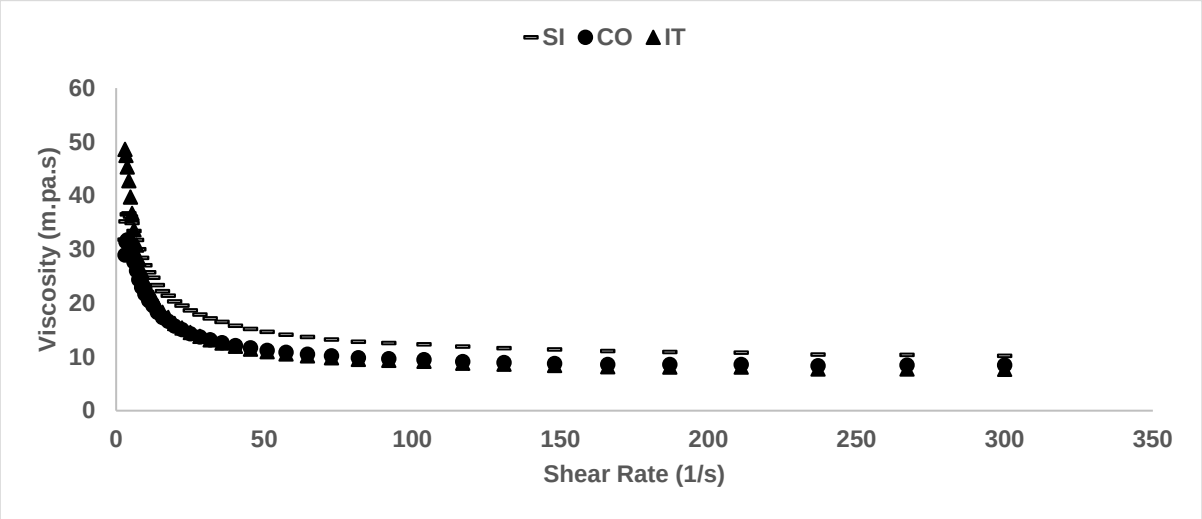

Figure 3 Viscosity curves of SI, IT and CO O/W Pickering emulsions.

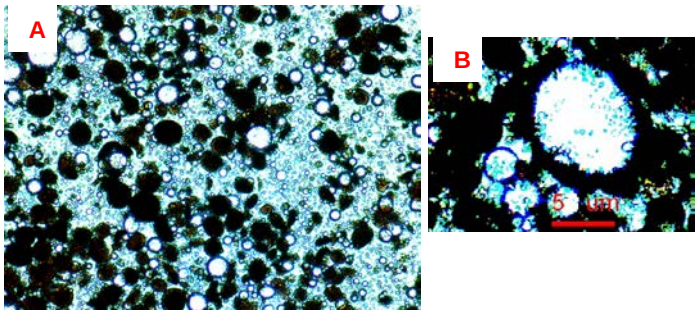

Figure 4 Optical microscope images of Pickering emulsions A)100 x, B)400 x.

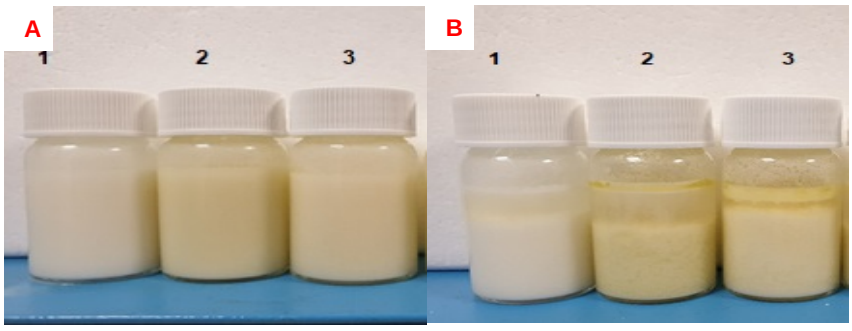

Figure 5: Images of the O/W emulsions at 0 day (A) and after 30 days of storage (B). (1-SI, 2-CO, 3-IT)

4. Conclusion

The results of this study on the effect of olive oil polyphenols on O/W Pickering emulsions stabilized by starch particles highlighted that Olive oil polyphenols significantly ($P<0.05$) affected the colloidal properties and the physical stability of the emulsified systems as they reduced the size of the oil particles and increased the physical stability towards destabilization phenomena. This study could open new paths for further investigations of the technological properties of olive oil polyphenols in o/w emulsions stabilized by Pickering mechanisms.

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